



Isolation of *Salmonella* from poultry droppings and other environmental sources in Awka, Nigeria

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Summary

Objective: A survey of *Salmonella* contamination of poultry droppings used as manure, retail fresh beef, fresh beef retailers' aprons and fresh beef retail tables, was carried out.

Design: A total of 120 samples of poultry droppings collected from five poultry farms, 96 fresh beef samples, 96 beef retailers' aprons and 96 fresh beef retail tables were examined for the presence of *Salmonella* species.

Results: Different *Salmonella* serotypes were isolated from all the sources. *Salmonella paratyphi* A had an isolation rate of 12.5% from poultry droppings, 4.2% from fresh beef, and 2.1% and 4.2% from meat retailers' aprons and tables respectively. Other serotypes isolated from the sources included *S. typhimurium*, *S. enteritidis*, *S. gallinarum*, *S. pullorum*, *S. typhi* and *S. agama*. *Salmonella typhi* was not isolated from poultry droppings throughout the survey.

Conclusion: There is a need to create more environmental and personal hygiene awareness among the Nigerian populace, especially among food vendors.

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Introduction

The genus *Salmonella* is one of the most common causes of food-borne infectious disease in the world.¹ A characteristic feature of this organism is its wide host range, which comprises most animal species including mammals, birds and cold-blooded animals in addition to humans.

A variety of food products, especially poultry and other types of meat products, are the most important sources of human *Salmonella* infection, but water-borne outbreaks have also occurred.^{1–3} Animals are mainly infected through feed, drinking water or environmental sources.^{4,5} The organism's route of infection is the faecal-oral route via food or water contaminated with faeces or urine of previously infected persons or animals.^{6–8}

A number of studies in Nigeria have shown that *Salmonella* infections, especially the enteric fever

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(typhoid and paratyphoid fever), is endemic in many parts of the country.^{8–11} The wide host range, the large number of convalescent and chronic healthy carriers and environmental sources in the communities increase the reservoir status of Salmonella infection and enhance its endemicity, especially in areas with low environmental hygiene. Animal droppings have been shown to be a potential reservoir for many enteric organisms.¹²

In Nigeria, poultry droppings are extensively used as manure for the cultivation of crops. The application of poultry droppings to land provides nutrients for the crop's growth as well as organic matter for soil conditioning and this can pose a danger to public health especially when the crops are eaten raw. The frequency of reports of typhoid and paratyphoid fever in Nigeria and especially in Awka is becoming worrying. There is a continual need to search for other environmental sources that could be important in the spread of typhoid and paratyphoid fever in Nigeria, especially in Awka.

The present study aims to isolate Salmonella from poultry droppings used for manure, from raw beef, the tables used for selling raw beef and the aprons worn by the fresh meat sellers. This is intended to help elucidate some of the environmental sources of Salmonella infections in Awka, Nigeria.

Materials and methods

Sample collection

All the samples were collected within the Awka Metropolis. Awka is the capital of Anambra State in Nigeria.

Poultry droppings were collected from five poultry farms once every week for six months between March and August 2002. Sterile spatulas were used to collect samples of freshly passed poultry droppings in sterile universal sampling bottles. The droppings were collected from litter at random points and transported to the laboratory where they were analysed within one hour from the time of collection. A total of 120 samples of poultry droppings were collected and analysed.

Meat (beef) samples were collected from four different fresh meat (beef) retailers in Eke Awka market in sterile wide mouth bottles with caps. Sterile cotton wool swab sticks dipped in sterile, normal saline were used to swab and collect samples from the exposed surfaces of raw meat retail tables and the exposed sides of aprons worn by the meat retailers. The samples were then sent to the laboratory where they were analysed within one hour from the time of collection. A total of 96 fresh meat

samples, 96 swab samples of raw meat retail tables and 96 swab samples of raw meat retailers' aprons were collected and analysed.

Sample analysis

The isolation of Salmonella from poultry droppings was carried out according to the method described by Barrell⁴ and Collee et al.¹³ A sterilized tablespoon was used to introduce a spoonful (approximately 10 g) of poultry droppings into 500 ml flasks containing 200 ml of selenite F broth (LAB M, Bury, UK. Product code Lab 44A). The inoculated flasks were rotated for proper mixing and incubated for 24 h at 42 °C.¹⁴ Sub-cultures were thereafter made onto plates of deoxycholate citrate agar (DCA) (LAB M, Bury, UK. Product code Lab 29) and propylene glycol deoxycholate agar (PGDA). The plates were incubated at 37 °C for 24 h. Salmonella-typical colonies on the plates (pale or colorless with or without black centers on DCA and a bright red color on PGDA) were cultured onto triple sugar iron agar (TSIA) (LAB M, Bury, UK. Product code Lab 53) slants, urea agar (Oxoid m53), lysine broth and incubated at 37 °C for 24 h. Suspected Salmonella colonies that were hydrogen sulfide positive on TSIA, urease negative and lysine positive were purity checked by sub-culturing onto fresh PGDA plates. The purity checked colonies were transferred to agar slants by streaking and from there further biochemical tests which included sugar fermentation, decarboxylase test, indole, methyl red, citrate utilization, potassium cyanide (KCN), vogesproskauer (VP) and glycerol fermentation were carried out to identify them.^{13,14} Serological characteristics of the isolates were investigated by the slide agglutination test using commercial polyvalent O, H and specific antisera (Difco).¹⁵ Overnight cultures from nutrient agar were used to perform the biochemical and serological tests.

Raw meat (beef) samples (25 g) were sliced into small pieces using a sterile scalpel and forceps on sterile metal trays and introduced into flasks containing 200 ml of nutrient broth and incubated for 24 h at 37 °C. The pre-enriched cultures were then transferred into flasks containing 200 ml of selenite F broth and incubated for 24 h at 42 °C.¹⁴ The cultures were then plated on DCA and PGDA. The plates were incubated at 37 °C for 24 h. Typical Salmonella colonies were then identified as described above.

Swabs of the retail tables and the aprons worn by the meat retailers were each used to inoculate 200 ml of nutrient broth in culture bottles and incubated for 24 h at 37 °C. The pre-enriched cultures were then transferred into flasks containing

Table 1 Different *Salmonella* serotypes and rates of their isolation from poultry droppings and other sources.

Serotypes	Poultry droppings	Raw beef	Beef retailers' aprons	Beef retailers' tables
	No. (%) Positive	No. (%) positive	No. (%) positive	No. (%) Positive
<i>S. paratyphi</i> A	15 (12.5)	4 (4.2)	2 (2.1)	4 (4.2)
<i>S. typhimurium</i>	8 (6.7)	2 (2.1)	1 (1)	4 (4.2)
<i>S. enteritidis</i>	6 (5)	0	0	0
<i>S. gallinarum</i>	8 (6.7)	2 (2.1)	2 (2.1)	2 (2.1)
<i>S. pullorum</i>	5 (4.1)	0	0	0
<i>S. typhi</i>	0	8 (8.3)	6 (6.2)	10 (10.4)
<i>S. agama</i>	0	2 (2.1)	0	1 (1)
Other serotypes	4 (3.3)	6 (6.2)	4 (4.2)	6 (6.2)
Total	46 (38.3)	24 (25)	15 (15.6)	26 (27)
No. of samples examined	120	96	96	96

200 ml of Selenite F broth. The flasks were incubated for 24 h at 42 °C. The cultures were then plated on DCA and PGDA and incubated for 24 h at 37 °C. Typical *Salmonella* colonies were then identified as described earlier.

Results

The different *Salmonella* serotypes and their rate of isolation from poultry droppings, raw beef, raw beef retailers' aprons and tables are shown in Table 1. Many of the *Salmonella* serotypes isolated from all the sources are known to be pathogenic to man. *Salmonella paratyphi* A was isolated from 12.5% of the poultry droppings, from 4.2% of fresh beef, and from 2.1% and 4.2% of meat retailers' aprons and tables respectively. *Salmonella pullorum* and *Salmonella enteritidis* were isolated only from poultry droppings. *Salmonella typhi* was not isolated from poultry droppings but was isolated from raw beef (8.3%), meat retailers' aprons (6.2%) and meat retailers' tables (10.4%). *Salmonella agama* was neither isolated from poultry droppings nor from meat retailers' aprons but was isolated at a very low rate from beef (2.1%) and beef retailers' tables (1%).

Table 2 Prevalence (%) of different *Salmonella* serogroups isolated from poultry droppings and other sources.

Sample source	Serogroups					
	A	B	C	D	Others	Total
Poultry droppings	12.5	6.7	0	15.8	3.3	38.3
Raw beef	4.2	4.2	0	10.4	6.2	25
Apron swabs	2.1	1	0	8.3	4.2	15.6
Table swabs	4.2	4.2	0	12.5	6.1	27

The prevalence (%) of different *Salmonella* serogroups isolated from all the sample sources is shown in Table 2. No *Salmonella* in serogroup C was isolated from any of the sources. *Salmonella* in serogroup D was isolated more than the *Salmonella* in other serogroups.

Discussion

Different *Salmonella* serotypes in different serogroups were isolated from all the sample sources examined in this study. Many of the *Salmonella* serotypes isolated are known to be pathogenic to man.

In Nigeria, especially in many parts of Anambra state, poultry droppings are sold to farmers at the rate of about N100 (1\$) per bag of 10–15 kg. The farmers use the poultry droppings as a good source of manure for the cultivation of crops and vegetables. The use of poultry droppings for the cultivation of crops serves the dual purposes of enriching the soil for improved crop yields and economically disposing of the droppings. However, the addition of the poultry droppings directly into soils without any form of treatment poses some public health problems since they contain pathogenic microorganisms. The pathogenic microorganisms can contaminate the surrounding crops and vegetables and become a source of infection, especially when such crops or vegetables are eaten raw or brought home where they can contaminate other materials. The pathogenic microorganisms can also be discharged onto surfaces that are used for drinking after run-off during rainfall.

A variety of food products including meat have been shown to be important sources of human *Salmonella* infections.^{3,4} In the present study, fresh beef, beef retail tables and aprons worn by meat retailers contained several *Salmonella*

serotypes (Table 1), many of which are known to be pathogenic to man. It was observed during sample collection that the table surfaces used for meat retail were very rough and contained small gullies and crevices which probably arose from machete cuts given to it when the beef was being cut for customers. The tables and the aprons are rarely washed or sanitized before fresh batches of beef are received. It was therefore not surprising that almost identical Salmonella serotypes isolated from the beef were also isolated from the tables and aprons. It was also observed that the tables were not covered up after the day's sales. The tables could therefore be a source of the spread of Salmonella to other materials by flies and by direct contact with human hands.

More Salmonella serotypes were isolated from poultry droppings than from other sources examined. Although the sources of microbial contamination were not determined, it is possible that the droppings may have been contaminated by the Salmonella that contaminated the feeds and water given to the birds. Chuaheng and Yeoh¹⁶ noted that soil and fecal materials on the feathers and feet of birds constitute a major source of microbial contamination of poultry carcasses.

The observations made in the present study may also be factors associated with the high frequency and endemicity of typhoid and paratyphoid fever in many parts of Nigeria. The rate of occurrence of enteric fever in many parts of Nigeria now causes some authors and medical practitioners to question the integrity of the Widal test, the routine serodiagnostic test for enteric fever in Nigeria.^{8,11,17}

In conclusion, there is a need to create more environmental and personal hygiene awareness among the Nigerian populace, especially among food vendors. Many food vendors do not know that there is a close relationship between personal and environmental hygiene and the spread of disease-causing microorganisms.

Conflict of interest: No conflict of interest to declare.

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